FILE 'HOME' ENTERED AT 07:09:11 ON 17 JAN 2002

FILE 'MEDLINE, AGRICOLA, CAPLUS, BIOSIS, EMBASE, WPIDS' ENTERED AT
07:09:18 ON 17 JAN 2002

L1 19375 S (CYSTEINE OR CYSTINE) W (PROTEASE OR PROTEINASE OR PEPTIDAS
L2 34 S L1 (S) BACILLUS
L3 15 DUP REM L2 (19 DUPLICATES REMOVED)
L4 14 S L3 NOT PY>2000

=>

TED DATA FROM 14 ANSWERS CONTINUE? Y/N:y

L4 ANSWER 1 OF 14 MEDLINE
ACCESSION NUMBER: 2001190517 MEDLINE
DOCUMENT NUMBER: 20530068 PubMed ID: 11079699
TITLE: Formation of biogenic amines in raw milk Hispanico cheese manufactured with proteinases and different levels of starter culture.
AUTHOR: Fernandez-Garcia E; Tomillo J; Nunez M
CORPORATE SOURCE: Departamento de Tecnologia de Alimentos, INIA, Madrid, Spain.. fgarcia@inia.es
SOURCE: JOURNAL OF FOOD PROTECTION, (2000 Nov) 63 (11) 1551-5.
Journal code: C48; 7703944. ISSN: 0362-028X.
PUB. COUNTRY: United States
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 200104
ENTRY DATE: Entered STN: 20010413
Last Updated on STN: 20010413
Entered Medline: 20010405

AB Two proteinases, a neutral proteinase from **Bacillus** subtilis and a **cysteine proteinase** from Micrococcus sp., were used to accelerate the ripening process of raw cow's milk Hispanico cheese, a semihard variety. Two levels (0.1% and 1%) of a commercial starter culture containing Lactococcus lactis subsp. lactis and L. lactis subsp. cremoris were added for cheese manufacture. The influence of both factors, proteinase addition and level of starter culture, on the growth of amino acid-decarboxylating microorganisms and on the formation of biogenic amines during cheese ripening was investigated in duplicate experiments. The population of tyrosine decarboxylase-positive bacteria, which represented less than 1% of the total bacterial population in most cheese samples, and tyrosine decarboxylase-positive lactobacilli was not influenced by proteinase addition or level of starter culture. Tyramine was detected in all batches of cheese from day 30. Its concentration was significantly ($P < 0.05$) influenced by proteinase addition but not by the level of starter culture and increased with cheese age. After 90 days of ripening, 103 to 191 mg/kg of tyramine was found in the different cheese batches. Histamine was not detected until day 60 in cheese with neutral proteinase and 1% starter culture and until day 90 in the rest of the cheeses. The concentration of this amine did not exceed 20 mg/kg in any of the batches investigated. Phenylethylamine and tryptamine were not found in any of the samples.

L4 ANSWER 2 OF 14 MEDLINE
ACCESSION NUMBER: 2000145388 MEDLINE
DOCUMENT NUMBER: 20195888 PubMed ID: 10733250
TITLE: Effect of added proteinases and level of starter culture on the formation of biogenic amines in raw milk Manchego cheese.
AUTHOR: Fernandez Garcia E; Tomillo J; Nunez M
CORPORATE SOURCE: Departamento de Tecnologia de Alimentos, INIA, Madrid, Spain. fgarcia@inia.es
SOURCE: INTERNATIONAL JOURNAL OF FOOD MICROBIOLOGY, (1999 Nov 15) 52 (3) 189-96.
Journal code: AVJ; 8412849. ISSN: 0168-1605.
PUB. COUNTRY: Netherlands
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 200004
ENTRY DATE: Entered STN: 20000427
Last Updated on STN: 20000427
Entered Medline: 20000420

AB The influence of two proteinases (**Bacillus** subtilis neutral proteinase and Micrococcus sp. **cysteine proteinase**) and two starter culture levels (0.1% and 1%) on biogenic amine formation has been studied in raw ewes' milk Manchego cheese. Amino acid decarboxylating micro-organisms were determined on tyrosine enriched selective media. Biogenic amines were analysed by capillary electrophoresis in citrate buffer at pH 3.6. Addition of proteinases and level of starter culture did not influence the population of micro-organisms with amino acid decarboxylating activity, which represented on average 1% of the bacterial population in 30-day-old cheeses. Tyramine and histamine were detected in all batches of cheese from day 30. Concentrations of tyramine and histamine were higher in cheeses made from milk with neutral proteinase (up to 356 and 284 mg kg(-1), respectively, after 90 days) than in cheeses made from milk with **cysteine proteinase** (up to 269 and 189 mg kg(-1), respectively) or with no proteinase added (up to 305 and 226 mg kg(-1), respectively). Formation of tyramine and histamine was also favoured in cheeses made with 1% starter culture with respect to cheeses made with

only 0.1% starter culture, probably due to the higher pH values of the former cheeses. After 90 days of ripening, concentrations of 10-20 mg kg(-1) phenylethylamine were observed in 9 of the 12 batches, and levels < 10 mg kg(-1) tryptamine were only detected in 3 batches, with no significant relationship between the concentration of these amines and proteinase addition or level of starter culture.

L4 ANSWER 3 OF 14 MEDLINE
ACCESSION NUMBER: 1999216336 MEDLINE
DOCUMENT NUMBER: 99216536 PubMed ID: 10196127
TITLE: The crystal structure of pyroglutamyl peptidase I from **Bacillus** amyloliquefaciens reveals a new structure for a **cysteine protease**
AUTHOR: Odagaki Y; Hayashi A; Okada K; Hirotsu K; Kabashima T; Ito F; Yoshimoto T; Tsuru D; Sato M; Clardy J
CORPORATE SOURCE: Department of Chemistry and Chemical Biology, Cornell University, Ithaca, NY 14853-1301, USA.
CONTRACT NUMBER: CA24487 (NCI)
SOURCE: STRUCTURE WITH FOLDING & DESIGN, (1999 Apr 15) 7 (4) 399-411.
Journal code: DEB; 100889329. ISSN: 0969-2126.
PUB. COUNTRY: ENGLAND: United Kingdom
Journal; Article; JOURNAL ARTICLE
LANGUAGE: English
FILE SEGMENT: Priority Journals
OTHER SOURCE: PDB-1AUG
ENTRY MONTH: 199906
ENTRY DATE: Entered STN: 19990618
Last Updated on STN: 20000407
Entered Medline: 19990610

AB BACKGROUND: The N-terminal pyroglutamyl (pGlu) residue of peptide hormones, such as thyrotropin-releasing hormone (TRH) and luteinizing hormone releasing hormone (LH-RH), confers resistance to proteolysis by conventional aminopeptidases. Specialized pyroglutamyl peptidases (PGPs) are able to cleave an N-terminal pyroglutamyl residue and thus control hormonal signals. Until now, no direct or homology-based three-dimensional structure was available for any PGP. RESULTS: The crystal structure of pyroglutamyl peptidase I (PGP-I) from Bacillus amyloliquefaciens has been determined to 1.6 A resolution. The crystallographic asymmetric unit of PGP-I is a tetramer of four identical monomers related by noncrystallographic 222 symmetry. The protein folds into an alpha/beta globular domain with a hydrophobic core consisting of a twisted beta sheet surrounded by five alpha helices. The structure allows the function of most of the conserved residues in the PGP-I family to be identified. The catalytic triad comprises Cys144, His168 and Glu81. CONCLUSIONS: The catalytic site does not have a conventional oxyanion hole, although Cys144, the sidechain of Arg91 and the dipole of an alpha helix could all stabilize a negative charge. The catalytic site has an S1 pocket lined with conserved hydrophobic residues to accommodate the pyroglutamyl residue. Aside from the S1 pocket, there is no clearly defined mainchain substrate-binding region, consistent with the lack of substrate specificity. Although the overall structure of PGP-I resembles some other alpha/beta twisted open-sheet structures, such as purine nucleoside phosphorylase and cutinase, there are important differences in the location and organization of the active-site residues. Thus, PGP-I belongs to a new family of cysteine proteases

L4 ANSWER 4 OF 14 MEDLINE
ACCESSION NUMBER: 1998038838 MEDLINE
DOCUMENT NUMBER: 98038838 PubMed ID: 9344414
TITLE: Inhibition, reactivation, and determination of metal ions in membrane metalloproteases of bacterial origin using high-performance liquid chromatography coupled on-line with inductively coupled plasma mass spectrometry.
AUTHOR: Leopold I; Fricke B
CORPORATE SOURCE: Department of Stress and Developmental Biology, Institute of Plant Biochemistry, Weinberg 3, Halle, 06120, Germany.
SOURCE: ANALYTICAL BIOCHEMISTRY, (1997 Oct 15) 252 (2) 277-85.
Journal code: 4NK; 0370535. ISSN: 0003-2697.
PUB. COUNTRY: United States
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199711
ENTRY DATE: Entered STN: 19971024
Last Updated on STN: 20000303
Entered Medline: 19971121

AB High-performance liquid chromatography coupled on-line with inductively coupled plasma mass spectrometry (HPLC-ICP-MS) was used for the characterization of metal ions in several metalloproteases of bacterial origin. The different components of the bacterial extracts were separated on a size-exclusion column. The eluent of the HPLC system was continuously

transported to the ICP-MS system for rapid, reproducible, and sensitive analyses of trace elements in the metalloproteases. Two different membrane proteases from **Bacillus** cereus and Pseudomonas aeruginosa were characterized to be zinc metalloproteases using enzymological methods and HPLC-ICP-MS. The zinc content was determined to be three molecules of zinc per protein molecule for the B. cereus protease and one molecule of zinc per protein molecule for the P. aeruginosa protease. For another purified protease, a periplasmic alanyl aminopeptidase of P. aeruginosa, the lack of protein bound metal ions could be clearly determined-a confirmation that this main aminopeptidase of P. aeruginosa belongs to the **cysteine protease** family. The presence of nonionic detergents can influence the distribution of trace elements during the HPLC separation. Therefore, the use of these substances should be avoided during enzyme purification for metal analyses or they should be exchanged later for zwitterionic and ionic detergents with more strongly dissociating properties.

L4 ANSWER 5 OF 14 MEDLINE
ACCESSION NUMBER: 97321861 MEDLINE
DOCUMENT NUMBER: 97321861 PubMed ID: 9178563
TITLE: Purification and characterization of a dipeptidyl carboxypeptidase from Pseudomonas sp. WO24.
AUTHOR: Ogasawara W; Abe N; Hagio T; Okada H; Morikawa Y
CORPORATE SOURCE: Department of Bioengineering, Nagaoka University of Technology, Niigata, Japan.
SOURCE: BIOSCIENCE, BIOTECHNOLOGY, AND BIOCHEMISTRY, (1997 May) 61 (5) 858-63.
Journal code: BEP; 9105717. ISSN: 0916-8451.
PUB. COUNTRY: Japan
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: B
ENTRY MONTH: 199707
ENTRY DATE: Entered STN: 19970812
Last Updated on STN: 20000303
Entered Medline: 19970731

AB A dipeptidyl carboxypeptidase (DCP) activity was detected in cell-free extracts of Pseudomonas sp. WO24. After purification and characterization the enzyme was found to be homogeneous by SDS-PAGE, and had a molecular mass of 74,000 Da by SDS-PAGE and 72,000 Da by gel filtration, indicating that it is monomeric. The isoelectric point was 5.2 and optimum pH was 6.5-7.0. It showed a specific activity of 780 mumol/min/mg, which is the highest of the values shown by known enzymes. The enzyme hydrolyzed angiotensin I to angiotensin II and sequentially released Phe-Arg and Ser-Pro from the C-terminus bradykinin. The DCP could not cleave imido-bonds, Gly-Gly bonds, or tripeptides. The enzymatic activity was completely inhibited by 0.001 mM EDTA and 0.1 mM O-phenanthroline, but it was not affected by general serine and **cysteine protease** inhibitors. Addition of Zn2+ completely restored the original activity of the inactivated DCP treated with EDTA. These results suggest that this enzyme is a zinc metalloprotease. The characteristics of the purified enzyme are slightly different from those of the DCPs from Escherichia coli, Pseudomonas maltophilia, and Corynebacterium equi, and considerably from those of the DCP from **Bacillus** pumilus.

L4 ANSWER 6 OF 14 MEDLINE
ACCESSION NUMBER: 95260306 MEDLINE
DOCUMENT NUMBER: 95260306 PubMed ID: 7741709
TITLE: A pepstatin-insensitive aspartic proteinase from a thermophilic Bacillus sp.
AUTHOR: Toogood H S; Prescott M; Daniel R M
CORPORATE SOURCE: Thermophile Research Unit, University of Waikato, Hamilton, New Zealand
SOURCE: BIOCHEMICAL JOURNAL, (1995 May 1) 307 (Pt 3) 783-9.
Journal code: 9YO; 2984726R. ISSN: 0264-6021.
PUB. COUNTRY: ENGLAND: United Kingdom
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199506
ENTRY DATE: Entered STN: 19950615
Last Updated on STN: 20000303
Entered Medline: 19950606

AB **Bacillus** sp. strain Wp22.A1 produced a cell-associated aspartic proteinase which was purified to homogeneity using phenyl-Sepharose (hydrophobic and affinity chromatography) and Mono Q. The proteinase has a molecular mass of 45 kDa by SDS/PAGE and a pI of 3.8. It is insensitive to pepstatin, but is sensitive to the other aspartic proteinase-specific inhibitors diazoacetyl-DL-norleucine methyl ester (DAN) and 1,2-epoxy-3-(p-nitrophenoxy)propane. Inactivation by DAN was only partial, suggesting that it had non-specifically modified an aspartate residue at a

site other than the active site. The enzyme was not inhibited by any of the serine or **cysteine proteinase** inhibitors tested. Maximum proteolytic activity was observed at pH 3.5. The proteinase had a higher activity with haemoglobin, but was more specific (Vmax./Km) for cytochrome c. Substrate inhibition was observed with both these substrates. The cleavage of oxidized insulin B chain tended to occur at sites where the P1 amino acid was bulky and non-polar, and the P1' amino acid was bulky and polar, such as its primary cleavage site of Val2-Asn3. The proteinase was stable in the pH range 2.5-5.5. Thermostability was increased in the presence of Ca2+, although to a lesser extent at higher temperatures. The thermostabilities at 60, 70, 80 and 90 degrees C were 45 h, 102, 21 and 3 min respectively in the presence of Ca2+

L4 ANSWER 7 OF 14 MEDLINE
ACCESSION NUMBER: 89025675 MEDLINE
DOCUMENT NUMBER: 89025675 PubMed ID: 3052431
TITLE: A bacterial factor induces changes in cysteine proteinase forms in the cellular slime mould Dictyostelium discoideum.
AUTHOR: North M J
CORPORATE SOURCE: Department of Biological Science, University of Stirling, Scotland, U.K.
SOURCE: BIOCHEMICAL JOURNAL, (1988 Aug 15) 254 (1) 269-75.
Journal code: 9YO; 2984726R. ISSN: 0264-6021.
PUB. COUNTRY: ENGLAND: United Kingdom
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 198811
ENTRY DATE: Entered STN: 19900308
Last Updated on STN: 20000303
Entered Medline: 19881122

AB The electrophoretic pattern of cysteine proteinases in axenically grown myxamoebae of Dictyostelium discoideum can be altered by the addition of either Gram-negative (Klebsiella aerogenes, Escherichia coli) or Gram-positive (Micrococcus lysodeikticus, **Bacillus** subtilis) bacteria to the culture. No changes occurred, however, if either yeast or latex beads were used in place of bacteria. The changes involved the simultaneous loss of proteinases characteristic of the axenic cells (the A-forms) and the acquisition of those found in cells which have been grown on bacteria (the B-forms). Using K. aerogenes the conversion was complete within 4 h. Extracellular proteinase activity was unaffected during this period. After the D. discoideum cells had been lysed, no equivalent change in proteinase band pattern could be produced either by prolonged incubation of cell extracts or by treatment with proteinases. An identical conversion could be induced in cultures of myxamoebae by a factor, **cysteine proteinase** converting factor (CPCF), present in the 15,000 g supernatant of a sonicated suspension of K. aerogenes. CPCF was macromolecular, as demonstrated by both ultrafiltration and gel filtration, acid-precipitable, but was soluble in ethanol or alkali. Its activity was unaffected by treatment with trypsin. The results suggested that CPCF might be a component of the bacterial cell wall, and since its activity was affected by lysozyme treatment, peptidoglycan is implicated. The results can be interpreted in terms of a novel nutrient-dependent post-translational change which affected most of the cysteine proteinases present in D. discoideum myxamoebae.

L4 ANSWER 8 OF 14 AGRICOLA
ACCESSION NUMBER: 1998:81477 AGRICOLA
DOCUMENT NUMBER: IND21644947
TITLE: An enzymatic analysis of the storage mite Lepidoglyphus destructor.
AUTHOR(S): Stewart, G.A.; Hage-Hamsten, M. van.; Krska, K.; Thompson, P.J.; Olsson, S.
CORPORATE SOURCE: University of Western Australia, Nedlands.
SOURCE: Comparative biochemistry and physiology. Part B, Biochemistry & molecular biology, Feb 1998. Vol. 119B No. 2. p. 341-347
Publisher: New York, NY : Elsevier Science Inc.
ISSN: 1096-4959
NOTE: Includes references
PUB. COUNTRY: New York (State); United States
DOCUMENT TYPE: Article
FILE SEGMENT: U.S. Imprints not USDA, Experiment or Extension
LANGUAGE: English

AB Extracts of Lepidoglyphus destructor were examined for the presence of digestive enzymes known to be allergenic in the pyroglyphid mites, such as Dermatophagoides pteronyssinus and D. farinae, with particular emphasis on the proteases and carbohydrases. Three serine proteases and one **cysteine protease** were detected, each with an apparent molecular weight of 25 K as judged by gel filtration. The serine proteases appeared to correspond to the trypsin, chymotrypsin and collagenolytic enzymes previously demonstrated in mites belonging to the genus

Dermatophagoides. Chromatofocusing studies showed that each of the serine proteases was polymorphic. Extracts of L. destructor were also found to contain amylase, glucoamylase and an enzyme(s) that lysed Gram-positive bacteria, such as Micrococcus lysodeikticus and **Bacillus** megaterium. These data indicate that extracts of L. destructor contain a spectrum of digestive enzymes similar to that shown to be present in the Pyroglyphid mites. The allergenicity of such enzymes in L. destructor remains to be determined.

L4 ANSWER 9 OF 14 AGRICOLA
ACCESSION NUMBER: 1998:39506 AGRICOLA
DOCUMENT NUMBER: IND21175433
TITLE: Characterization and distribution of chymotrypsin-like and other digestive proteases in Colorado potato beetle larvae.
AUTHOR(S): Novillo, C.; Castanera, P.; Ortego, F.
AVAILABILITY: CNAL (QL495.A7)
SOURCE: Archives of insect biochemistry and physiology, 1997. Vol. 36, No. 3. p. 181-201
Publisher: New York, N.Y. : Wiley Liss.
CODEN: AIBPEA; ISSN: 0739-4462
NOTE: Includes references
PUB. COUNTRY: New York (State); United States
DOCUMENT TYPE: Article
FILE SEGMENT: U.S. Imprints not USDA, Experiment or Extension
LANGUAGE English
AB Chymotrypsin-like, carboxypeptidase A-like and leucine aminopeptidase-like activities have been detected in the midgut of Colorado potato beetle larvae, Leptinotarsa decemlineata Say (Coleoptera: Chrysomelidae), in addition to the previously identified cathepsin B, D, and H. We have characterized a new chymotrypsin-like activity using the specific substrates N-succinyl-L-alanyl-L-alanyl-L-prolyl-L phenylalanine-p-nitroanilide and N-benzoyl-L-tyrosine p-nitroanilide. This novel proteinase, with a pH optimum of 6.5-6.5, was neither activated by thiol compounds nor inhibited by **cysteine proteinase** inhibitors. Among several serine proteinase inhibitors tested, PMSF was the most effective. Gelatin-containing SDS-PAGE gels and activity staining after gel electrophoresis indicated that chymotrypsin-like activity was associated with a major band of about 63 KDa and a minor band of about 100 KDa. The major exopeptidases found in the larval midgut extracts were leucine aminopeptidase and carboxypeptidase A. Most endo- and exoproteolytic activities studied were evenly distributed among the midgut sections, indicating that there is no clear regional differentiation in the digestion of proteins. Chymotrypsin and cathepsin B, D, and H were mainly located in the endoperitrophic and ectoperitrophic spaces, with only a small activity associated with the midgut epithelium. In contrast, leucine aminopeptidase was mainly located on the wall tissue, although some activity was distributed between the ecto- and endoperitrophic spaces. The potential roles of Colorado potato beetle digestive chymotrypsin in the proteolytic activation of the delta-endotoxin from **Bacillus** thuringiensis, and in the use of protease inhibitors to disrupt protein digestion, are discussed.

L4 ANSWER 10 OF 14 CAPLUS COPYRIGHT 2003 ACS
ACCESSION NUMBER: 2000:58691 CAPLUS
TITLE: Papers to Appear in Forthcoming Issues
AUTHOR(S): Anon.
SOURCE: Protein Expression Purif. (2000), 18(1), iv
CODEN: PEXPEJ; ISSN: 1046-5928
PUBLISHER: Academic Press
DOCUMENT TYPE: Journal; Miscellaneous
LANGUAGE: English
AB Prodn., Purifn., and Properties of an Extracellular Laccase from Rigidoporus lignosusMaria Teresa Cambria, Antonio Cambria, Santa Ragusa, and Enrico RizzarelliPurifn. and Characterization of Macrodontain I, a **Cysteine Peptidase** from Unripe Fruits of Pseudananas macrodontes (Morr.) Harms (Bromeliaceae)Laura M. I. Lopez, Cynthia Sequeiros, Claudia L. Natalucci, Adriana Brullo, Bruno Maras, Donatella Barra, and Nestor O. ZaffiniStaphylococcal Protein A as a Fusion Partner Directs Secretion of the El.alpha. and El.beta. Subunits of Pea Mitochondrial Pyruvate Dehydrogenase by **Bacillus** subtilisJ. Ignacio Moreno, Jan A. Miernyk, and Douglas D. RandallExtracellular Expression, Purifn., and Characterization of a Winter Flounder Antifreeze Polypeptide from Escherichia coliLi Tong, Qingsong Lin, W. K. Raymond Wong, Asma Ali, Daniel Lim, Wing L. Sung, Choy L. Hew, and Daniel S. C. YangVectors Allowing Amplified Expression of the Saccharomyces cerevisiae Gal3p-Gal80p Gal4p Transcription Switch: Applications to Galactose-Regulated High-Level Prodn. of ProteinsAlok Kumar Sil, Ping Xin, and James E. HopperExtremely Thermostable Elongation Factor G from Aquifex aeolicus: Cloning, Expression, Purifn., and Characterization in a Heterologous Translation SystemKirill A. Martemyanov, Anders Liljas, and Anatoly T. GudkovOptimization of Inclusion Body Solubilization and

Renaturation of Recombinant Human Growth Hormone from Escherichia coliAshok K. Patra, R. Mukhopadhyay, R. Mukhija, Anuja Krishnan, L. C. Garg, and Amulya K. Panda. (c) 2000 Academic Press.

L4 ANSWER 11 OF 14 CAPLUS COPYRIGHT 2002 ACS
ACCESSION NUMBER: 1997:194231 CAPLUS
DOCUMENT NUMBER: 126:195874
TITLE: Expression of a proteinase inhibitor and a Bacillus thuringiensis .delta.-endotoxin in transgenic poplars.
AUTHOR(S): Cornu, D.; Leple, J.C.; Bonade-Bottino, M.; Ross, A.; Augustin, S.; Delplanque, A.; Jouanin, L.; Pilate, G.
CORPORATE SOURCE: Station d'Amelioration des Arbres Forestiers, INRA, Ardon, F-45160, Fr.
SOURCE: For. Sci. (Dordrecht, Neth.) (1996), 49(Somatic Cell Genetics and Molecular Genetics of Trees), 131-136
CODEN: FOSCEH; ISSN: 0924-5480)
PUBLISHER: Kluwer
DOCUMENT TYPE: Journal
LANGUAGE: English
AB Genetic transformation has been used to improve poplar tolerance toward Chrysomela tremulae, a coleoptera causing severe damages in nurseries and young poplar plantations. We have shown in an in vitro assay that cysteine proteinase represent the major proteinase activity in the midgut of C. tremulae. Moreover, in this system the cysteine proteinase inhibitor OCI effectively inhibits most of the digestive proteinase activity. This proteinase inhibitor and the Bacillus thuringiensis .delta.-endotoxin CRY IIIA, also known to be active against coleptera, were both evaluated for their toxicity against C. tremulae. Transgenic poplars expressing either oci or cry IIIA gene were produced. Insects feeding on this transgenic poplars exhibit reduced larval growth, altered development and increased mortality when compared to the control.

L4 ANSWER 12 OF 14 CAPLUS COPYRIGHT 2002 ACS
ACCESSION NUMBER: 1990:527685 CAPLUS
DOCUMENT NUMBER: 113:127685
TITLE: Protease-deficient gram-positive bacteria and their use as host organisms for the production of recombinant products
INVENTOR(S): Blackburn, Peter; Lonetto, Michael Arthur; Chang, Edward L.; Polak, June
PATENT ASSIGNEE(S): Public Health Research Institute of the City of New York, Inc., USA
SOURCE: PCT Int. Appl., 31 pp.
CODEN: PIXXD2
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 8910976	A1	19891116	WO 1989-US1056	19890314
W: AU, DK, FI, HU, JP				
RW: AT, BE, CH, DE, FR, GB, IT, LU, NL, SE				
AU 8937651	A1	19891129	AU 1989-37651	19890314
AU 627918	B2	19920903		
EP 370103	A1	19900530	EP 1989-906977	19890314
R: AT, BE, CH, DE, FR, GB, IT, LI, LU, NL, SE				
HU 53154	A2	19910328	HU 1989-4054	19890314
JP 03500606	T2	19910214	JP 1989-506242	19890314
ZA 8902325	A	19900328	ZA 1989-2325	19890329
FI 9000045	A	19900104	FI 1990-45	19900104
DK 9000015	A	19900205	DK 1990-15	19900104
PRIORITY APPLN. INFO.:			US 1988-180483	19880505
			WO 1989-US1056	19890314

OTHER SOURCE(S): MARPAT 113:127685
AB **Bacillus** AP (NP- (alk. and neutral proteases-deficient **Bacillus**) free of residual protease activity, i.e. the residual serine protease (RSP and/or SH-dependent residual **cysteine protease** (RCP), is prepd. by site specific mutagenesis, e.g deletion mutation, of gene(s) encoding RSP and/or RCP. A method of screening Bacillus deficient in RSP and/or RCP, esp. from Bacillus AP-/NP- is given. The Bacillus mutants thus prepd. are useful in manufg. heterologous proteins.

L4 ANSWER 13 OF 14 BIOSIS COPYRIGHT 2002 BIOSIS
ACCESSION NUMBER: 1997:178512 BIOSIS
DOCUMENT NUMBER: PREV199799470225
TITLE: Effects of lectins, CRY1A/CRY1B Bt delta-endotoxin, PAPA, protease and alpha-amylase inhibitors, on the development of the rice weevil, Sitophilus oryzae, using an artificial seed bioassay.

AUTHOR(S): Pittendrigh, B. R.; Huesing, J. E.; Shade, R. E.; Murdock, L. L.
CORPORATE SOURCE: Dep. Entomol., 1158 Entomol. Hall, Purdue Univ., West Lafayette, IN 47907-1158 USA
SOURCE: Entomologia Experimentalis et Applicata, (1997) Vol. 82, No. 2, pp. 201-211.
ISSN: 0013-8703.
DOCUMENT TYPE: Article
LANGUAGE: English

AB An artificial maize seed bioassay was developed to evaluate potential resistance factors against the rice weevil, Sitophilus oryzae. Weevils reared in artificial seeds compared to those reared in whole maize seeds: (i) developed faster, (ii) had similar within seed developmental mortalities, (iii) were lighter in weight upon emergence and (iv) oviposited the same number of eggs. Using this bioassay we found that E-64, a **cysteine protease** inhibitor, decreased the number of emerged adults per seed and delayed within seed developmental time, suggesting that the rice weevil utilizes a **cysteine protease** to digest its dietary protein. Weevils fed inhibitors of trypsin and chymotrypsin, Bowman-Birk and Kunitz inhibitors respectively, developed normally. Para-amino-L-phenylalanine (PAPA), a non-protein amino acid implicated as an insect resistance factor in Vigna vexillata, was lethal at dietary levels of 0.2% (w/w) and higher. An extract from Amaranthus caudatus seeds delayed the developmental time of the rice weevil at dietary levels of 0.2% (w/w) and increased mortality at dietary levels of 1.0% (w/w). Several proteins tested, including Griffonia simplicifolia agglutinin II, phytohemagglutinin extract containing common bean alpha-amylase inhibitor, pokeweed agglutinin, **Bacillus** thuringiensis CRY1A/CRY1B endotoxin, and an alpha-amylase inhibitor from wheat, had no effect on the rice weevil. The artificial maize seed bioassay was adapted by pelleting the seed for use with an ultrasonic insect feeding monitor to determine the feeding activity of rice weevils as they developed from egg hatch to pupation.

L4 ANSWER 14 OF 14 WPIDS COPYRIGHT 2002 DERWENT INFORMATION LTD
ACCESSION NUMBER: 2001-007053 [01] WPIDS
DOC. NO. CPI: C2001-001720
TITLE: A catalytic antagonist useful for specific targeting of an effector molecule comprises a targeting moiety that specifically binds to the target molecule.
DERWENT CLASS: B04 C06 D16
INVENTOR(S): BOTT, R R; DAVIS, B G; ESTELL, D A; JONES, J B; SANFORD, K J
PATENT ASSIGNEE(S): (GEMV) GENENCOR INT INC
COUNTRY COUNT: 92
PATENT INFORMATION:

PATENT NO	KIND	DATE	WEEK	LA	PG
WO 2000064485	A2	20001102	(200101)*	EN	144

RW: AT BE CH CY DE DK EA ES FI FR GB GH GM GR IE IT KE LS LU MC MW NL OA PT SD SE SL SZ TZ UG ZW
W: AE AG AL AM AT AU AZ BA BB BG BR BY CA CH CN CR CU CZ DE DK DM DZ EE ES FI GB GD GE GH GM HR HU ID IL IN IS JP KE KG KP KR KZ LC LK LR LS LT LU LV MA MD MG MK MN MW MX NO NZ PL PT RO RU SD SE SG SI SK SL TJ TM TR TT TZ UA UG US UZ VN YU ZA ZW
AU 2000046595 A 20001110 (200109)

APPLICATION DETAILS:

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NOVELTY - A catalytic antagonist (I) of a target molecule comprises a targeting moiety that specifically binds to the target molecule. The targeting moiety is attached to an enzyme that degrades the target molecule to reduce binding of the target molecule to its cognate ligand and targeting molecule, resulting in the release of the antagonist, allowing it to bind and degrade another target molecule.
DETAILED DESCRIPTION - INDEPENDENT CLAIMS are also included for the

following:

(1) degrading a targeting molecule comprising contacting the target molecule with (I);

(2) an enzyme (II) having an altered substrate specificity comprising a targeting moiety attached to a subsite comprising the substrate binding site of the enzyme;

(3) directing the activity of an enzyme to a specific target comprising providing (II) and contacting with the target, where the enzyme specifically binds to the target, localizing the activity of the enzyme at the target;

(4) enhancing the activity of a drug that acts as an inhibitor of a receptor or an enzyme comprising coupling a serine hydrolase to the drug so that when the drug binds the receptor or the enzyme the serine hydrolase degrades the receptor or enzyme; and

(5) inhibiting an enzyme or a receptor comprising contacting the enzyme or receptor with a chimeric molecule comprising a ligand that binds the enzyme or receptor attached to an enzyme that degrades the cognate ligand of the enzyme or receptor.

USE - (I) can be used as catalytic antagonist for specific targets.

ADVANTAGE The effector molecule of (I) is transported directly to the sight of action by the targeting moiety of (I).

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